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Molecular Identification of the Na^+-Activated K^+ Channel

Progress in understanding sodium-activated potassium channels (K_{Na}), suggested to function in excitable cells both during physiological conditions and protectively during hypoxia, has been limited by their unknown molecular identity. In this issue of *Neuron*, Salkoff and coworkers now show that members of the *Slo* gene family, *Slo2.1* and *Slo2.2*, encode functional K_{Na} channels.

Sodium-activated potassium channels (K_{Na}) were originally described in inside-out patch recordings from guinea pig ventricular myocytes (Kameyama et al., 1984). A few years later, expression of K_{Na} channels in vertebrate neurons was confirmed using excised inside-out patch recordings (Dryer et al., 1989; Haimann et al., 1990), and these channels appear to be quite widespread throughout the nervous system. K_{Na} channels typically have a relatively high unitary conductance, comparable to that of BK type Ca^{2+}-activated and ATP-sensitive K^+ channels, and K_{Na} should therefore produce significant effects on cell physiology when it becomes active. Nevertheless, almost from the beginning, the most important questions about the properties and significance of neuronal K_{Na} channels have been controversial. This controversy has centered around two closely related questions—under what conditions do these channels become active, and what role do they play in either the normal or abnormal physiology of excitable cells?

The initial descriptions of K_{Na} channels in cardiac myocytes and neurons suggested that relatively high concentrations of Na^+, typically in the range of 35 to 80 mM, are required to evoke significant channel activation, at least in excised membrane patches. Consequently, it has not been easy to see how these channels can become active during the course of normal spike discharge. Moreover, at least some of the macroscopic recordings that have supported a role for K_{Na} channels in shaping spike waveform have been flawed by inadequate voltage clamp (Dryer, 1991). Several solutions to this problem have been proposed. For example, diffusion barriers, such as those within the restricted space of dendrite processes and axonal nodes (Koh et al., 1994; Safronov and Vogel, 1996), could allow local Na^+ concentrations to build up to the necessary levels, especially during a train of action potentials (Schwindt et al., 1989) or if an array of K_{Na} channels were tightly clustered around individual Na^+ channels (Koh et al., 1994). In addition, it is possible that K_{Na} channels are more sensitive to Na^+ in intact cells than they are in excised patches (Rodrigo, 1993), possibly owing to interactions with other ions (see further below) or due to interactions with some other diffusible intracellular factor. In this regard, "run down" of K_{Na} gating is observed in some but not all cell types, and it is likely from this and other observations that K_{Na} represents a *class* of channels (Dryer, 1994).

Clearly, the issue of Na^+ sensitivity is closely tied to the physiological or pathophysiological role of K_{Na} channels. For example, a relatively low Na^+ sensitivity would suggest that the major role of K_{Na} is to protect neurons during ischemia or other metabolic stress (Dryer, 1994), and it is generally accepted that K_{Na} channels play such a role in cardiac myocytes. However, an increase in Na^+ sensitivity or accumulation by one or more of the mechanisms listed above appears necessary in order for K_{Na} channels to play a direct role in normal spike discharge or synaptic transmission.

Progress in understanding the nature and role of K_{Na} has been severely limited because its molecular identity is unknown. Now, Yuan et al. (2003) in this issue of *Neuron* report that K_{Na} is encoded by members of the *Slo* gene family, *Slo2.1* and *Slo2.2*; the latter of these was named *Slack* when it was originally isolated (Joiner et al., 1998). The *Slo2* genes are closely related to *Slo1*, which encodes large-conductance Ca^{2+}-activated K^+ channels, and *Slo3*, which encodes a pH-sensitive K^+ channel. The Salkoff laboratory previously studied a *C. elegans* ortholog of *Slo2* and found that its gating was dependent on intracellular Cl^- and Ca^{2+} ions (Yuan et al., 2000). (The Ca^{2+} dependence of the *C. elegans Slo2* ortholog is interesting because these organisms do not appear to express voltage-activated Na^+ channels.) While the mammalian Slo2.2 channel does not *require* Cl^- for activity in *Xenopus* oocytes, this anion can facilitate channel activation if Na^+ is present; indeed, the actions of Na^+ and Cl^- are highly synergistic. This cooperativity may be physiologically important, as Slo2.2 channels can become active at normal physiological levels of Na^+ if sufficient Cl^- is present. This feature of K_{Na} channels has not been described previously, and it will be interesting to see if it holds true for native K_{Na} channels. However, other aspects of the mammalian Slo2 channels expressed in heterologous systems are remarkably similar to those of native K_{Na} channels described in most previous studies. These features include a relatively low basal sensitivity to Na^+, insensitivity to Li^+, a high unitary conductance that has a dependence on K^+ concentration significantly greater than what would be predicted from constant-field considerations, and the presence of multiple and prominent subconductance states.

Mammals appear to express two closely related *Slo2* genes, *Slo2.1* and *Slo2.2*, which are 96% similar within the regions encompassed by the six presumptive membrane-spanning domains. These two forms have a different tissue distribution, as *Slo2.1* is expressed in brain, heart, skeletal muscle, and many other tissues, whereas *Slo2.2* transcripts predominate in brain and are expressed in the heart at low levels. Indeed, the Slo2.2 gene product appears to be expressed at highest levels in olfactory bulb mitral cells, which also have a very high density of functional K_{Na} channels. By analogy to other K^+ channel gene families, it seems likely that functional K_{Na} channels in brain are heteromultimers, which could

explain some of the diversity in the properties of K_{Na} channels apparent in the current literature, especially if the gating of Slo2 channels is modified by β subunits. In this regard, there is already evidence that Slo2 channels can modify the gating properties of Slo1 channels when they are coexpressed in heterologous systems (Joiner et al., 1998).

An important feature of the article by Yuan et al. (2003) is the observation that *C. elegans* mutants lacking functional Slo2 channels are more sensitive to hypoxic stress. Ischemia typically causes an increase in intracellular Na^+, Ca^{2+}, and Cl^-, which would cause activation of K_{Na} and hyperpolarization. This in turn would be expected to inhibit action potential discharge and improve Ca^{2+} transport. Both of these effects should be protective for nerve and muscle, and it is quite reasonable to hypothesize that this role is conserved over a wide range of species and tissues.

The identification of genes encoding mammalian K_{Na} channels will make it possible to establish their physiological roles through powerful genetic and biochemical methods that have proved invaluable for other families of channels. It will certainly enhance efforts to identify agents that selectively modify K_{Na} gating, which could be useful therapeutic agents. It will also make it possible to determine the structural and evolutionary relationship of K_{Na} to other K^+ channels and to Na^+-dependent cation channels. Activation of *any* channel with a unitary conductance of 40–200 pS will have major consequences for an excitable cell. This landmark study will markedly accelerate progress on K_{Na} channels, which for nearly two decades has been "slo" and frustrating.

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Selected Reading

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Bassoon's Part in Two Presynaptic Orchestras

The protein Bassoon is found in the cytoskeletal matrix at the active zone of conventional synapses and in presynaptic ribbons of photoreceptor synapses. Two new studies in *Neuron* show that Bassoon's most prominent role at conventional synapses is to enable vesicle cycling, whereas, in photoreceptors it attaches the ribbon to the presynaptic membrane.

The new technique of electron microscopy applied to the central and peripheral nervous system in the middle 1950s to early 1960s had a profound effect on our understanding of synapses. The big news was, of course, that presynaptic structures contained clouds of tiny vesicles that were clearly the basis for the finding of Bernard Katz and his colleagues that transmitter is released in multimolecular packets. But there was more that the electron microscope showed. At many central synapses, fuzzy material, perhaps interacting with synaptic vesicles, seemed to be stuck to the cytoplasmic face of the presynaptic membrane (schematized in Figure). With suitable staining methods, the fuzz resembled pyramids each about 60 nm in diameter and 100 nm apart, linked together by filaments to form a hexagonal web.

Not all synapses looked the same. A few sorts of neuron, including photoreceptors, were shown to possess unique presynaptic structures in the form of vesicle-covered plates, or ribbons, oriented orthogonal to the presynaptic membrane and apparently glued to it by a region called the "arciform density" (see Figure). The two obvious questions posed by these presynaptic structures concerned their constituent components and the role these played in synaptic transmission. For decades, one could be excused for thinking that these questions had been forgotten. That has all changed now.

The low contrast and small dimensions of the presynaptic web of central synapses seen in the electron microscope contributed to a lingering doubt about its reality that has only recently been dispelled. A turning point came when Phillips et al. (2001) purified presynaptic webs and, amazingly, showed that they could be reconstituted in vitro. Not only was it now pretty certain that the web was real, but it was also apparent that it extended from the cytoplasm at the presynaptic terminal to the presynaptic membrane and clear across to the postsynaptic membrane. An indirect boost to the credibility of presynaptic webs in central neurons came from a study of the presynaptic structures of the neuromuscular junction (Harlow et al., 2001). Though the two structures are rather different, electron microscopy had revealed, as in central neurons, a fuzzy material on the presynaptic membrane associated with vesicles. Careful reconstruction from electron tomography showed that the fuzzy material was actually a symmetrical skeleton of ribs, pegs, and beams connecting vesicles to calcium channels and, one imagines, acting as a machine to organize transmission.

The molecular composition of the presynaptic structures at the neuromuscular junction is largely unknown,